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Hypoderma siculum sp. nov. from Italy

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Abstract — *Hypoderma siculum* is described and illustrated as a new species from southeast Sicily (Italy) that occurs on remnants of *Ferula communis* (*Apiaceae*). Its ecology and taxonomic and phylogenetic relationships are discussed.

Key words — morphology, taxonomy, ITS phylogeny

Introduction

Field investigation of the *Ascomycetes* of Sicily occurring on decaying remnants of *Ferula communis* (Lantieri 2009) revealed a new *Hypoderma* species, described here as *Hypoderma siculum*.

Materials & methods

Collections of the new species were made between 2007 and 2010 at an elevation of 700 m a.s.l. in southeast Sicily. Morphological and microscopic examinations were carried out on fresh material and on dried specimens rehydrated in water. Observations and measurements were made in water and Melzer's reagent. Ascus and ascospore size ranges from the holotype were based on 50 measurements, using an Optika optical microscope (model BK 1301), with 40× or 100× (oil immersion) objectives. All voucher specimens were deposited in the fungarium of the Royal Botanic Gardens, Kew K(M) and in the fungal reference collection of Landcare Research in New Zealand (PDD).

DNA was extracted separately from two sets of four fruiting bodies taken from each of two separate blackened areas on leaf pieces within the isotype specimen (PDD 99894), using REDExtract-N-Amp Plant PCR Kits (Sigma, USA). The perithecia were

Table 1. Specimens used in phylogenetic analysis

Species	Country of origin, host	Collection voucher	GenBank acc. no.
Coccomyces australis	Argentina, *Desfontainia spinosa*	ICMP 16772	EF191241
Hypoderma commune	Sweden, ?*Euphorbia* sp.	Hanson 2006-451 (UPS)	JF690769 *
H. cordylines	New Zealand, *Cordyline australis*	ICMP 17344 (ex-holotype culture)	JF683421 *
	New Zealand, *Phormium* sp.	ICMP 17359	JF683420 *
H. hederae	Scotland, *Hedera helix*	Lantz & Minter 421 (UPS)	JF690770 *
H. rubi	China, host unknown	unknown	GU138735
	China, host unknown	unknown	GU138736
	China, host unknown	unknown	GU138738
	China, host unknown	unknown	GU138739
	China, host unknown	unknown	GU138741
	China, host unknown	unknown	GU138743
	China, host unknown	unknown	GU138745
	China, host unknown	unknown	GU138746
	China, host unknown	unknown	GU138750
	China, host unknown	unknown	GU138751
	China, host unknown	unknown	GU367895
	China, host unknown	unknown	GU367898
	New Zealand, *Coprosma* sp.	ICMP 18768	JF683417 *
	New Zealand, *Melicytus ramiflorus*	ICMP 18325	JF 683418 *
	New Zealand, *Pseudopanax* sp.	ICMP 17349	JF683416 *
	New Zealand, *Rubus cissoides*	ICMP 17339	JF 683419 *
	Sweden, *Rubus lindebergii*	Hanson 2002-230 (UPS)	JF690771 *
H. siculum	Italy, *Ferula communis*	PDD 99894 (isotype)	JF683424 *
H. stephanandrae	China, *Stephanandra chinensis*	unknown	GU138753
H. vincetoxici	Sweden, *Vincetoxicum hirundinaria*	Lantz 405 (UPS)	JF690772 *
Lophodermium agathidis	New Zealand, *Agathis australis*	ICMP 18327	JF683423 *
	New Zealand, *Metrosideros fulgens*	ICMP 17345	JF683422 *
L. eucalypti	New Zealand, *Leptospermum scoparium*	ICMP 16796	EF191235
L. gamundiae	Argentina, *Nothofagus dombeyi*	ICMP 16797	EF191239

* = newly generated for this study).
UPS = Uppsala University; ICMP = International Collection of Microorganisms from Plants (maintained by Landcare Research)

ground in extraction buffer with a plastic pestle in the Eppendorf tube. Following this, DNA extraction and PCR were carried out following manufacturer's instructions. ITS sequences were obtained from each extract following the methods of Johnston & Park (2005).

Using ClustalW (Thompson et al. 1994), our newly generated sequences were aligned with sequences deposited in Genbank as *Hypoderma rubi* or those that closely matched *H. rubi*. Because of the paucity of available data, ITS sequences were generated from several additional *Hypoderma* spp. from New Zealand, with DNA extracted from cultures grown from germinating ascospores. Other taxa included in the analysis were members *Lophodermium eucalypti*/*Coccomyces tumidus* clade recognized by Lantz et al. (2011) that formed a sister relationship to the core *Hypoderma* clade, and *Lophodermium agathidis* as the outgroup.

The taxa included are listed in Table 1, along with the Genbank accession numbers of our newly generated sequences. A Bayesian phylogenetic analysis was performed using MrBayes 3.1.2 (Huelsenbeck & Ronquist 2001; Ronquist & Huelsenbeck 2003) with gaps treated as missing data, using the K80+I+G model which was selected using the AIC method in MrModelTest 2.3 (Nylander 2004). The data set was run with 2 chains for 10 million generations, trees sampled every 1000 generations with a burn-in of 25%. Bayesian posterior probabilities were obtained from 50% majority rule consensus trees.

Taxonomy

Hypoderma siculum Lantieri, P.R. Johnst. & Medardi **sp. nov.** Plates 1–2

MycoBank MB 563145

Ascomata erumpentia inter amplas variiformes nigras areas, haud compositas, supra plantae superficiem, haud consociata cum zonae lineis. Partes nigrae ex 1–2 stratis hypharum parietibus obscuris et inflatis, formantibus amplum stratum, 5–8 µm latum sub plantae cuticula, constitutae. Aspectus in superficie: ascomata 1–1.5 × 0.5–0.8 mm, ellipsoidea vel leviter fusiformia vel navicularia si secta, extremitatibus repente rotundatis vel acutis, paene mersa in contextu plantae. Ascomata clausa parietibus nigrescentibus, sed plus minusve obscure griseis ad margines prominentes fissurae. Ascomata aperta per fissuram longitudinalem, se extendentem in totam longitudinem. Labra dilute griseola adsunt. Hymenium griseum caesio colore suffusum. Labri cellulae hyalinae ex hyphis inconstanter cylindricis, inflatis in nonnullis partibus, pariete crassa, cellulis 1 (raro 2) –septatis, 20–25 × 2 µm, mersis in involucro gelatino, constitutae. In recta media parte stroma tegens crassum usque ad 50 µm prope ascomatum centrum, sed tenuior ad margines atque tenens stroma basis ex strato inferiore et obscure brunneo texturae epidermoideae constitutum. Externa pars parietum cellularum stromatis tegentis obtecta materia densa, nigra, plus minusve granosa, ex particulis sine structura cellularum manifesta, sed nonnullis cellulis obscure polygonalibus, efformata. Excipulum abest. Subhymenium 20–40 µm crassum, ex textura intricata, hyphis 3–4 µm latis, parietibus tenuibus et hyalinis constitutum. Stroma basis 5–15 µm crassum, cum 3–4 stratis hypharum hyalinarum, 3–4 µm latarum, parietibus obscure brunneis et leviter inflatis, formantibus texturam intricatam. Ascosporae fusiformes vel cylindrico-fusiformes, nonnullae leviter curvae, 27–30 × 3–3.5 µm, laeves, hyalinae, haud septatae, guttulis haud compositis praeditae, saepe absentibus prope centrum, cinctae involucro gelatino, laxo, circiter 3 µm crasso. Asci clavato-stipitati, (85–)90–100 × 9–10 µm, apice rotundato, haud amyloidei, 8–sporigeri; sporae dispositae in parte superiore. Paraphyses numerosae, filiformes, 1–2 µm latae, emergentes usque ad 30 µm supra ascos, curvae, crispatae vel valde plicatae ad apicem, nonnullis parvis guttulis sparsis per totam longitudinem, sine septis. Specimina cum conidiis haud notata. Habitat: supra reliqua putrescentia Ferulae communis*; inventum solum hieme.*

Type: Italy. Sicily, vic. Vittoria (Ragusa), Riserva Naturale Orientata "Pino d'Aleppo", on decaying remnants of *Ferula communis*, 30/12/2007, Leg. Angela Lantieri (**holotype**, K(M) 167515; **isotype**, PDD 99894, GenBank JF683424).

Conidiomata not observed. Ascomata develop among large, irregularly shaped blackened areas on the plant surface, not associated with zone lines. The blackened areas have 1–2 layers of hyphae with walls dark and thick forming a 5–8 µm wide layer beneath the plant cuticle. In surface view ascomata 1–1.5 × 0.5–0.8 mm, elliptical to slightly fusiform or navicular in outline, with sharply rounded to acute ends, semi-immersed in the plant tissues. Closed ascomata with blackish walls, but more or less dark greyish near the prominent edges of the slit. Ascomata opening by a longitudinal slit, which extends along the whole length. Lips present, pale greyish. Hymenium grey with bluish reflexes. Lip cells hyaline, composed of irregularly cylindrical, in some points enlarged, thick-walled, 1 (rarely 2) –septate, cells, 20–25 × 2 µm embedded in a gelatinous sheath. In median vertical, covering stroma up to 50 µm thick near the centre of the ascomata, becoming thinner towards the edges, extending to the basal stroma, consisting of a inner layer of dark brown textura epidermoidea. Outer part of the walls of the cells in the covering stroma with dense, more or less granulose black matter, composed of small particles with no visible cellular structure, but some of them obscurely polygonal. Excipulum absent. Subhymenium 20–40 µm thick, composed of textura intricata, hyphae 1.5–2 µm diam. with hyaline, thin walls. Basal stroma 5–15 µm thick, comprises 3–4 layers of hyphae 3–4 µm diam. with walls dark brown and slightly thickened, forming a textura intricata. Ascospores fusiform or cylindrical-fusiform, some slightly curved, 27–30 × 3–3.5 µm, smooth, hyaline, aseptate, containing irregular oil drops often lacking near the middle, surrounded by loose gelatinous sheath about 3 µm thick. Asci clavate-stipitate, (85–)90–100 × 9–10 µm, apex rounded, not bluing in iodine, 8-spored, spores confined to the upper part. Paraphyses abundant, filiform, 1–2 µm diam., protruding up to 30 µm beyond the asci, curved, curled or remarkably bent at the apex, with some small oil-drops scattered along the length, without septa.

Habitat: On decaying remnants of *Ferula communis*, found only in winter.

Additional specimens examined: **ITALY**. **Sicily**, vic. Vittoria (Ragusa), Riserva Naturale Orientata "Pino d'Aleppo", 13/03/2008, [Leg. A. Lantieri] (K(M) 167516); 06/02/2009, [Leg. A. Lantieri] (K(M) 167517); 13/02/2010, [Leg. A. Lantieri] (K(M) 167518).

Discussion

Hypoderma siculum falls within the *Hypoderma* sensu stricto clade of Lantz et al. (2011). Genetic relations within this clade are poorly resolved in the ITS gene tree (Fig. 3). Despite this, *H. siculum* is genetically distinct from other named isolates within the clade, and this genetic isolation and its distinct

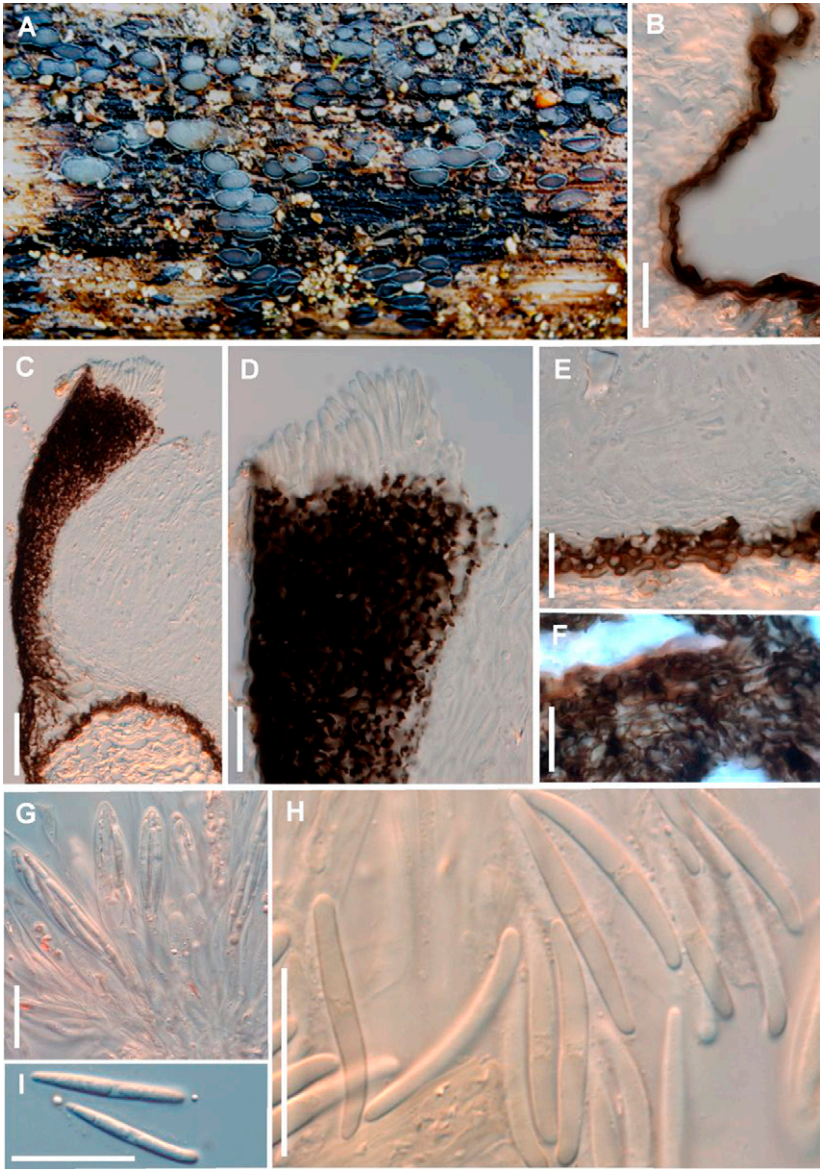

Plate 1. *Hypoderma siculum* (Holotype, K(M) 167515). A. fresh ascomata in situ; B. stromatic tissue surrounding ascomata, vertical section; C. upper wall of ascoma, vertical section; D. detail of lip cells, vertical section; E. detail of lower wall of ascoma, vertical section; F. detail of lower wall of ascoma, squash mount; G. asci and ascospores; H. ascospores in KOH; I. ascospores in water showing gelatinous sheath. Scale bars: A = 1 mm; B, D–H = 20 µm; C = 50 µm.

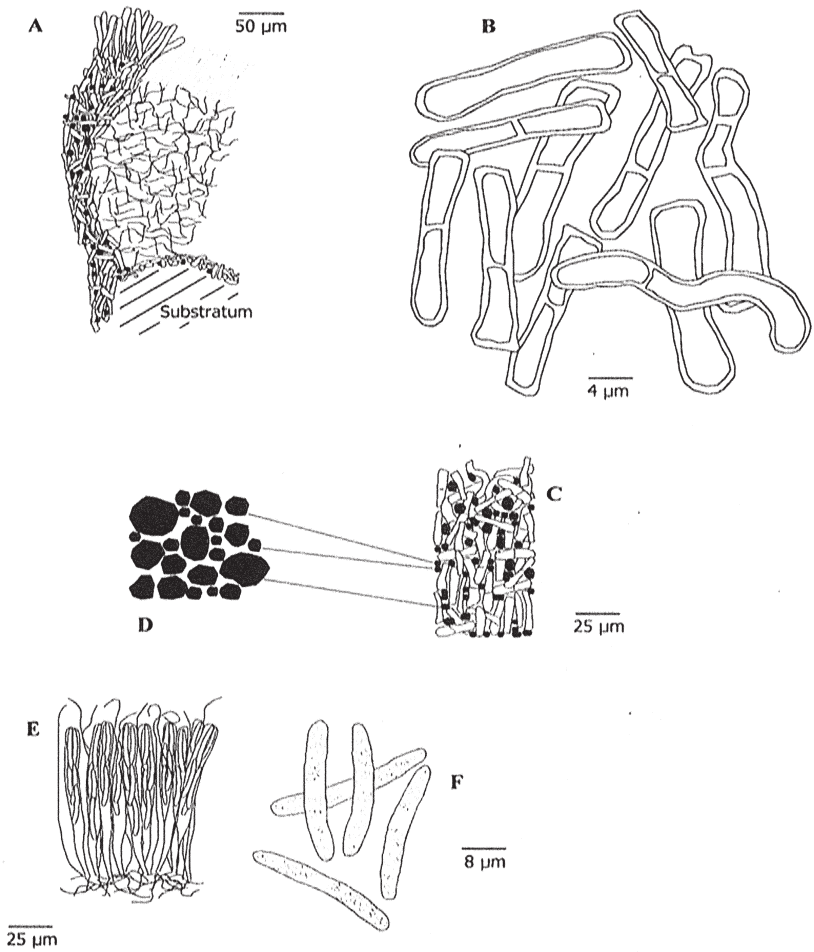

Plate 2. *Hypoderma siculum* (Holotype, K(M) 167515).
A. margin of ascoma in vertical section; B. stromatal cells; C. section of stroma; D. granulose matter; E. asci and ascospores; F. ascospores.

biology supports recognizing the fungus as an independent species. Other morphologically distinct taxa within this clade, such as *Hypoderma cordylines*, are also poorly resolved. More intensive, multi-gene phylogenies of Australasian species have shown that ITS alone does not adequately resolve relationships within this clade (unpublished data). The weak quality of the DNA extracted from the *H. siculum* dried specimens did not permit reliable sequencing of single copy genes.

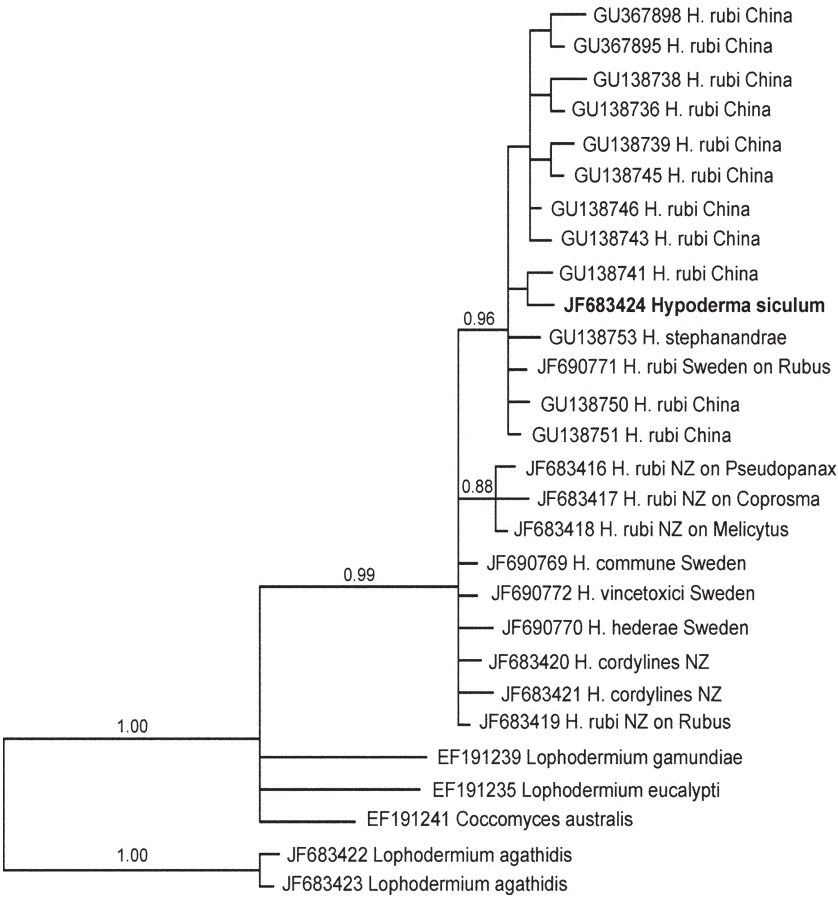

Figure 3. 50% majority-rule consensus phylogenetic tree based on Bayesian analysis of *Hypoderma siculum* and related taxa derived from ITS gene sequences. Bayesian posterior probabilities greater than 90% are shown above the edges. *Sclerotinia sclerotiorum* was selected as the outgroup.

Ferula communis L. (*Apiaceae*), a typical herbaceous plant of the Mediterranean area, is commonly associated with two *Hypoderma* species, *H. ferulae* Lantieri (Lantieri 2009) and *H. siculum*. Early reports of *Hypoderma rubi* (Pers.) DC. and *H. commune* (Fr.) Duby on *F. communis* (Duby 1862; Petrak 1943) are difficult to assess, as neither report is supported by specimens. It is uncertain in what sense the authors were using these names and impossible to check whether the specimens they reported match our *Ferula*-specialised species.

Extensive collecting in Sicily revealed only *H. ferulae* and *H. siculum* on *Ferula*. Scalia (1900: 37) reported *H. commune* from Sicily on a species in another genus of *Apiaceae*, *Thapsia garganica* L.; the report included no description of the fungus, and again there is no material available to verify the record. There is no evidence from collections made in Sicily that *H. siculum* occurs on other genera of *Apiaceae* (unpubl. data).

Hypoderma siculum is distinguished from *H. ferulae* by ascospore size and septation (21–24 × 2.5–3 µm and often 1–septate in *H. ferulae*), by the presence of an extended area of stromatic subcuticular tissue surrounding the ascomata, the colour of the hymenium when fresh (yellow-honey with greenish tinges in *H. ferulae*), and the lack of an excipular layer.

Hypoderma siculum resembles *H. rubi* morphologically, but as noted in the phylogenetic section, the application of this name by different authors is uncertain, and genetic relationships amongst specimens identified as *H. rubi* are poorly resolved. *Hypoderma rubi* sensu Powell (1974), Johnston (1990), and Medardi (2006) differs slightly from *H. siculum* in its ascospore size (20–25(–26.5) × 3–4 µm) and the fusiform-navicular ascospore shape. *Hypoderma commune* differs in having ascomata with yellow-greenish hymenium and smaller, non-septate spores (17–20 × 3–4 µm; Ellis & Ellis 1988).

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